ALTERNATIVE LARVAL DIETS AND GENERATIONAL TIME FOR GULF OF MEXICO BLENNY SPECIES

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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The purpose of this study was to analyze the effectiveness of nematodes (*Panagrellus redivivus* and *Turbatrix aceti*), *Artemia*, and rotifers (*Brachionus plicatilis*) as diet options for larval *Scartella cristata* to see how well nematodes could perform compared to the 'accepted' general diet of *Artemia* and rotifers. Four different diets, three consisting of nematodes, were to be tested in this endeavor to better increase larval performance and production value of this limited commercial species. Previous research, although limited and among varied species, produced mixed success for larval performance on nematode-based diets. Larval performance would be analyzed based on length-weight and RNA:DNA ratios. This information would help define an exact feeding regimen for this species which could translate to other similar blenny species for use in aquarium aquaculture. It may also further support the applicability of nematodes as a diet replacement for other aquacultured fish in the commercial realm. Unfortunately no breeding ever occurred over the study period.

ACKNOWLEDGMENTS (optional)

This project would not have been possible without the support of Dr. Ron I Eytan, Texas Sea Grant, the TAMUG Sea Life Facility, and volunteer efforts. I would particularly like to thank Jeff Plumlee, Josh Carter, Brett Mayberry Cole Chavez, Bridget Janowiak, Logan Bellingham, and the SLF volunteer staff for their contributions in collecting, building, and maintaining fish and food cultures over the last half year.

CHAPTER I

INTRODUCTION

The marine aquarium trade is evolving in response to an increase in aquaculture and changing preferences of hobbyists (Rhyne & Tlusty, 2012). This has significantly raised the demand for reef associated fish (Livengood & Chapman, 2007) and aquaculture has grown to fill the void of declining natural stocks due to wild over-collections (Murray & Watson, 2014). Sustainability of wild populations should be the goal for any aquarist (Livengood & Chapman, 2007), which is why optimum culture methods must be devised for all species possible within the aquarium trade. Reef fish, such as blennies, have already been proven to be successfully aquacultured and are available through aquarium fish dealers and hobbyists (Murray & Watson, 2014; Wingerter, 2012). Likewise, establishing breeding techniques for a single species has shown to be transferable to multiple similar species, as seen in Clownfish (*Amphiprion spp*.), Dottybacks (*Pseudochromis spp*.) and Blennies (Murray & Watson, 2014). In this regard, perfecting the breeding and rearing technique of one species could, through exploration of improving nutritional content of first feeds (Conceição, Grasdalen, & Rønnestad, 2003), increase the yield of not only that species, but other species that are similar as well.

The Molly Miller Blenny (*Scartella cristata*) was chosen for this study because of its relative abundance around the Gulf of Mexico (Grabowski, 2002), its current use in the aquarium trade, and its diverse diet (Mendes, Villaca, & Ferriera, 2009; Wingerter, 2012). The larvae are quite large, having a post-flexion length of 5.3mm total length (Ditty, Shaw, & Fuiman, 2005) and hatching at around 3mm (Eyberg, 1984; Wingerter, 2012). Molly Millers have been observed

feeding on nuisance algae, bacteria, and anemones in an aquarium setting, and could potentially be very valuable to the aquarium hobby for these reasons (Wingerter, 2012). They also have a similar diet to *Salarias spp.* (Mendes et al., 2009; Wilson, 2001), a fish already well established in the trade, and could potentially replace that species if their availability through aquaculture increases. Further improvement on culturing methods and marketing could lead to a higher availability and demand for Molly Miller Blennies.

Artemia spp. have long been used as the staple first or second food source for both marine and freshwater fishes (Lavens & Sorgeloos, 2000; Sorgeloos, Dhert, & Candreva, 2001). Global demand for live food has risen which has led to the need for new food types for use in aquaculture (Brüggemann, 2012). At the beginning of the 21st century *Artemia* demand was 800 metric tons per year, a majority of which comes from the Great Salt Lake in Utah (Sorgeloos et al., 2001). Yields from the lake can be affected by weather events like El Niño (Lavens & Sorgeloos, 2000; Sorgeloos et al., 2001) and it is also prone to overharvesting (Brüggemann, 2012). The *Artemia* themselves are hatched in a rather intensive process (Brüggemann, 2012; Sorgeloos et al., 2001) as opposed to other live feed alternatives, and have low lipid content, some indigestible amino acids, and are a larger size than alternatives (Conceição et al., 2003; Sorgeloos et al., 2001). Because of their large size, *Artemia* are generally fed in conjunction with Rotifers (Lim, Dhert, & Sorgeloos, 2003). Rotifers too have generally been accepted as a good first feed mainly due to their size (Anitha & George, 2006; Hundt et al, 2015; Lubenz, 1987), nutritional content, and easily achievable high densities (Lubenz, 1987). Rotifers are less demanding as culture animals but still can feed on live algae, which must be cultured in conjunction with the rotifers (Lubenz, 1987). They will accept inert foods although that brings

additional cost and decreases water quality at a faster rate. Although these staple foods exist and can be enriched (Sorgeloos et al., 2001) it is important that other live foods be explored which are less demanding to raise and have a potentially greater nutritional content.

Nematode species *Panagrellus redivivus* and *Turbatrix aceti* have been used as alternative food sources for fresh and saltwater fishes and invertebrates (Biedenbach, Smith, Thomsen, & Lawrence, 1989; Brüggemann, 2012; Hundt et al., 2015; Hofsten, Kahan, Katznelson & BarEl, 1983; Schlechtriem, Ricci, Focken, & Becker, 2004a). These nematodes are not parasitic, have no ability to defend against predation from fish larvae, and have large reproductive capabilities necessary to fulfill yields for larviculture (Brüggemann, 2012). They are both cultured in non-intensive processes with no reliance on cultured algal feed (Brüggemann, 2012; Hofsten et al., 1983; Ricci et al., 2004). The nematode *P. redivivus* has no ability to swim and must be artificially suspended, but *T. aceti* can swim throughout the water column (Brüggemann, 2012) making it easily accessible to larvae. The smaller *P. redivivus* has been reported from 50µm to 1700µm, comparable to rotifers (Anitha & George, 2006; Lim et al., 2003) and sizes for *T. aceti* range up to 2500µm (Brüggemann, 2012). Especially in the case of *P. redivivus*, emulsion in medications (Mohney, Lightner, Williams, & Bauerlein, 1990) and fatty acids (Schlectriem et al., 2004b) allows for incorporation of these materials into the tissue of the nematode. They can also be used in both fresh and saltwater interchangeably (Brüggemann, 2012), similar to *Artemia* and rotifers (*B. plicatilis*), by surviving long periods of time in either medium (Lim et al., 2003). This opens up the possibility of nematodes being ‘tailor-made’ to suit the needs of a particular species in a way *Artemia* and rotifers cannot.

This study will explore the effectiveness of nematode-based diets against a control diet of rotifers and *Artemia*. Traditional length-weight ratios will be used to determine larval fitness, as well as RNA:DNA ratios which have shown to be a good metric for larval health (Wright & Martin, 1985; Rooker, Holt, & Holt, 1997). This will also be coupled by a percent survival over a 25 day period before larval sacrifice. Time allowing, a secondary set of larvae will be raised through maturity in order to establish and exact generational time for this species. A nematode-based diet may prove to be as effective as or better than the control diet and reduce the overall culturing effort, allowing for larger yields of *S. cristata* larvae and potentially other related species of fish.

CHAPTER II

METHODS

Adult fish

Twenty (5M:15F) Adult Molly Miller Blennies (*Scartella cristata*) were collected from the South Jetty (Figure 1) in Port Aransas, Texas (27˚50'19.59"N, -97˚2'51.81"W) on November 15th 2015 after sundown using nets and barehanded tactics. The nets used were a baitwell net (2' handle, 8"x6"x6" net) and dipnet (5' handle, 13" D-frame net) but these were mainly used to corral Blennies that had already been caught by hand. Adult blennies (ranging 7-10+cm) in the infralittoral zone were cupped by hand and transferred to nets in most instances. Sex determination was done by referencing figures in past literature (Smith, 1974). Specimens were taken back to the Texas A&M Galveston Sea Life Facility the same night and acclimated to an existing system of four 20 gallon aquariums with a central sump roughly 20 gallons full. The reason for this over-collection was to protect against possible losses due to fighting, disease, or other unforeseen factors.

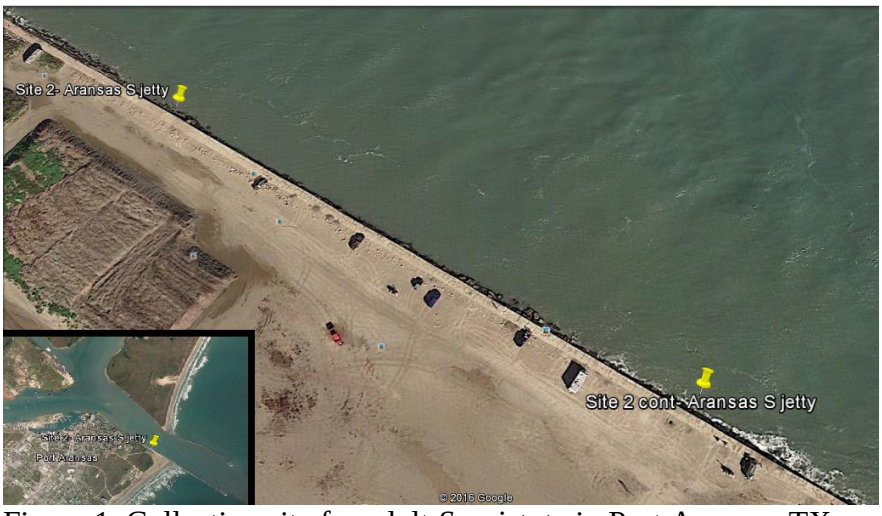

Figure 1. Collection site for adult *S. cristata* in Port Aransas, TX

Collected fish were partitioned into equal sections for an observation and quarantine period within the facility for one month before being introduced for breeding. After two initial mortalities (2F) twelve fish were partitioned individually within the four tank system (4M:2F:2F:4F) and the six remaining fish (1M:5F) were transferred to a 'mixer' table tank (5'x8'x5") to test if breeding would occur immediately without separate conditioning of the sexes. PVC, terracotta, and rock shelters were provided for each fish with an overabundance of shelter provided in the 'mixer' system. Aquarium conditions ranged from 19-27˚C and 32-36ppt salinity throughout the study period (Figure 2). Adult Blennies were fed a mixed diet of enriched frozen foods (brands San Francisco Bay Sally's, Omega One), live foods (adult *Artemia*), algae (*Spirulina spp.*), and home-made prepared foods modelled after natural diet structure (Mendes et al., 2009) to encourage breeding. The home-made diet was roughly 50:50 greens and animal protein. Introduction of the partitioned fish for breeding occurred on December 27th, 2015 with each male being paired with zero to two females based on aggression upon introduction. After 54 days within the four tank system all adults were transferred to the 'mixer' system on March 1st, 2016.

Cultured species

This study required the culturing of algae (*Nanochloropsis oculata*, *Isochrysis galbana*), rotifers (*Brachionus plicatilis*), brine shrimp (*Artemia salina*) and nematodes (*Panagrellus redivivus, Turbatrix aceti*) undertaken within the Sea Life Facility. Both algae species and the *Artemia* were available within the facility and culturing was managed by members of this and other projects. Rotifers were acquired from Reed Mariculture and both nematode cultures were acquired from a private dealer. The inert food 'Roti-Grow Plus' was also acquired from Reed

Mariculture as a food option for the rotifers. Both algae species were used as feed for the rotifers and *Artemia*. After January 10th, 2016 all live algae usage was replaced with 'RotiGrow Plus' due to unforeseen circumstances leading to algal cultures being unavailable. Rotifer cultures were maintained in five gallon pyramid hatchery cones available within the facility.

One nematode species (*T. aceti*) was raised in a vinegar solution (1:1 apple cider vinegar to water) and the other (*P. redivivus*) was cultured on an oatmeal medium. The oatmeal medium consisted of cooked whole oats with a sprinkling of active dry yeast. Both nematode species were housed in either mason jars or plastic takeout containers and covered with a secured cloth to prevent contamination. Nematode cultures were split monthly when densities became too high and rotifers were split every few days. These cultured organisms formed the basis of the four diets for the larvae.

Larval fish

The larval system was plumbed into the existing adult system above the four tanks and consisted of four plastic round tubs (16"x7") with a central mesh standpipe of 55μm. Water level was controlled by a Hartford loop which drained to a separate line leading to the sump. Larvae were to be fed four distinct diets: a control (Rotifer and *Artemia*) and three experimental diets (1. *T. aceti*, 2. *P. redivivus*, 3. Both nematode species) over a 25 day period before sacrifice. The process of tissue extraction for RNA:DNA ratios would follow the protocol established by Dr. Alvarado-Bremer using the "shake and stew" method (Alvarado-Bremer, Smith, Moulton, Lu, & Cornic, 2014).

The AUP for this study, IACUC 2015-0151, allowed for the use of 1000 fishes and that number was not exceeded. Wild fish were collected under permission from Texas Parks and Wildlife Scientific Research Permit No. SPR-1015-223.

CHAPTER III

RESULTS

Adult fish

No eggs were found to have been laid throughout the study period. Three adult mortalities occurred due to aggression between individuals. Aggressive biting of fins by other fish was observed for both sexes. This biting was sometimes exclusively against one individual, singled out regardless of tankmates, to the point where that individual died and the aggressive behavior of the other fish ceased. The increase of egg laying substrates into the tank enclosures seemed to limit aggression. Changes in coloration were also observed in most animals with a darkening/lightening of pigments or a uniform/barring pattern emerging based on stress levels from aggression or transferring, temperature changes, or other unknown factors. Individuals that appeared to be more dominant generally took on darker coloration and barring while individuals under stress lightened in color.

Cultured species

Rotifer and *Artemia* cultures were maintained originally on live algae and later on inert 'RotiGrow Plus' after January 15th, 2016 with no observable difference in performance of either organism. The 'RotiGrow Plus' did increase the need for culturing maintenance as dead algae cells coagulated and became too large for the rotifers and *Artemia* to consume. Both nematode species and rotifers were maintained at lower levels so as to reduce upkeep until needed for larval fish.

CHAPTER IV

DISCUSSION

Without any observed breeding over the study period it is not possible to draw any conclusion on the effectiveness of a nematode-based feeding regime for *S. cristata* at this time. What was observed was the behavior of adults which had not been factored into the original study design and thus no quantifiable data was collected. Future studies may benefit from monitoring the behavioral patterns of this species and the nuances of establishing pairings. This could reduce the number of targeted mortalities that occurred during this study when a single fish was attacked by all other tank mates until death. This would be especially pertinent as this species exhibits three distinct male phases (Neat et. al, 2003).

Grabowski (2002) found *S. cristata* spawning to occur from January to June based on back calculations from otolith aging, the timing of which coincided with the study period. An old measure of spawning time by Smith (1974) reported a mid-spring peak more towards April and a later fall peak, while another study showed that active breeding occured during the whole month of June (Neat, Locatello, & Rasotto, 2003). This wide range of spawning time would indicate that seasonality was not the reason for a lack of spawning behavior by the adult fish in this study. Neat *et. al* (2003) discuss alternative male tactics related to smaller 'sneaker' males, intermediate 'hole dweller' makes, and large 'nester' males. The size of the animals in this study would have classified them as 'nesters' according to Neat *et. al* (2003) which were the only size observed as being sexually active. They observed that both large and intermediate sizes lived in holes but there was no definition of just how big those holes were. The breeding substrate of PVC pipe and

terracotta pots inhabited daily by the adults in this study may not have matched the preferred breeding holes closely enough to induce egg laying.

Environmental conditions (Figure 2) were in no way grossly dissimilar from naturally fluctuating conditions along the Texas coastline where the blennies were collected. They may have been in fact too stable, as the adults never experienced any large swings in parameters that could be brought on by something like heavy rainfall or other weather conditions. However the seemingly year-long natural spawning of this species could imply that they would not need a key weather event in order to induce spawning, especially given their demersal laying pattern as opposed to group pelagic spawning. The model for the adult diet closely followed recently published data on the species (Mendes et al., 2009) matching available food products as close taxonomically as possible to published prey items. Feeding occurred as many as three times daily until satiation, which kept the adults well fed. For all of these reasons, there appears that some undetermined factor confounded the normal breeding of this species during the study, or that not enough time was allowed to induce breeding.

With 71 species represented through aquarium fish importation (Rhyne et al., 2012), Blenny species certainly remain a prime target for aquaculture efforts. Any identification of concerted breeding methodology or improvements of those methods should be met favorably by aquarists and scientists alike and lead to further advancements in aquaculture.

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APPENDIX A (optional)

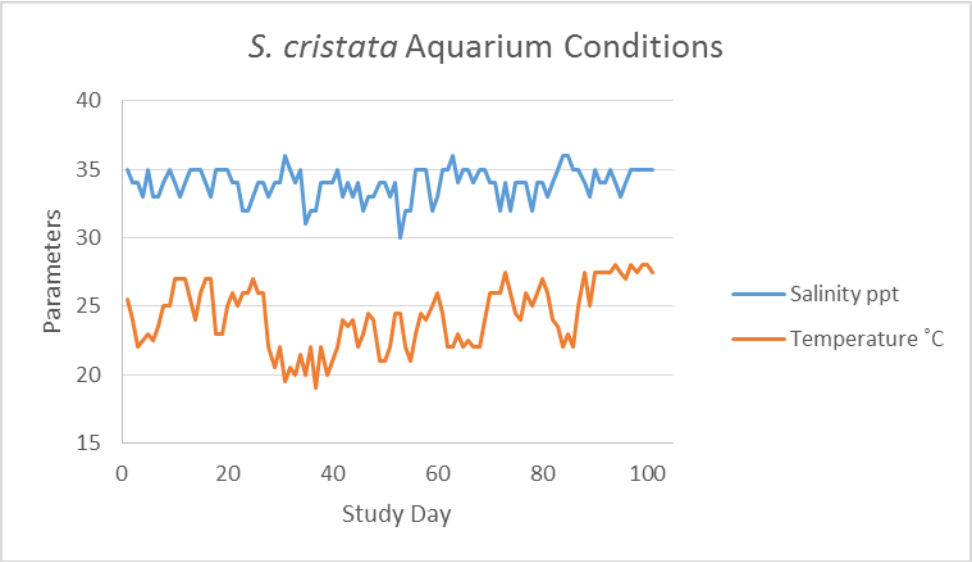

Figure 2. Trends of salinity and temperature (˚C) throughout the study period